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Retarded and Mosaic Phenotype in Regenerated Claw Closer Muscles of Juvenile Lobsters

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Abstract. The closer muscle in the paired claws of the lobster *Homarus americanus* become determined into their asymmetric form of a cutter and crusher type claw during the 4th and 5th juvenile stages and differentiate their fiber composition accordingly in subsequent juvenile stages. Our aim was to study the effects of claw loss during this critical juvenile period on muscle regeneration. Hence the fiber composition of the paired closer muscles in newly regenerated claws was examined histochemically following removal of both claws either in the 4th and 5th stages or in the 4th through 7th stages. The newly regenerated muscle was retarded compared to its original counterpart in both cases. In the former case, however, the retardation was temporary as the muscle composition in later stages resembled the original. Recovery in the latter was not apparent in later stages, suggesting that retardation is more permanent. Also in both protocols the newly regenerated closer muscle occasionally displayed a mosaic distribution, with slow fibers interspersed among fast fibers in a central band that is normally homogenously fast. Therefore, loss of the paired claws during a developmentally sensitive period affects the phenotype of the regenerated muscle with the change persisting for shorter or longer periods depending on how often the claws are lost.

Introduction

Crustaceans have an amazing ability of dropping an entrapped or endangered limb by breaking it off at a preformed fracture plane, thus allowing the animal to escape. Because such limb autotomy involves little loss of blood, the animal usually lives to regenerate a new limb. The ability to autotomize a limb varies not only among species, or within a species, but also within an individual, in that the chelipeds autotomize more readily than the walking legs. This is the case in lobsters (*Homarus americanus*) and particularly in their juvenile forms when a gentle pinch to the cheliped will result in autotomy whereas the walking limbs will need greater provocation. Indeed, lobsters with their solitary life-style and aggressive nature often lose claws in the wild and often lose them more than once.

Following the loss of a claw, a new one is regenerated which, in structure and function, resembles its predecessor. Although smaller in size initially, the regenerate limb grows over several molt cycles to assume pristine proportion at which time there is little to distinguish it from the original limb. A similar degree of fidelity applies internally, at least with muscles that regenerate the same fiber types as the original in the claw closer muscles in lobsters (Kent *et al.*, 1989), as well as in snapping shrimps (Govind *et al.*, 1986) and crayfish (Govind and Pearce, 1985).

A variation seen consistently in the newly regenerated closer muscle of the claw in crayfish and occasionally in the major claw of snapping shrimps was the appearance of a central band of fast fibers in a muscle that otherwise comprises 100% slow fibers. Because this regional distribution of fast and slow fibers is reminiscent of an early developmental stage in the closer muscle of crayfish and snapping shrimps, it was assumed that some aspects of ontogeny were recapitulated during regeneration. Such a variation in the phenotype did not persist, and the muscle assumed its pristine character over the next few molt cycles. These regenerative events were recorded in adult crayfish and shrimps where the muscle is fully differentiated. What would be the condition of regenerate muscles that had not yet differentiated their adult phenotype? We studied this question in the lobster *Homarus americanus*

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because we were familiar with the development of its closer muscle (Govind, 1984, 1989).

The paired claws and closer muscles in the lobster, *Homarus americanus*, become determined into a major and minor type early in juvenile development and subsequently differentiate their claw morphology and muscle fiber composition into their final form. While loss of both claws in the critical juvenile stages delays the determination of claw asymmetry to a later stage, more prolonged loss suppresses asymmetry altogether (Govind and Pearce, 1989). With such clear-cut effects of claw loss on the determination of asymmetry, it seemed likely that muscle regeneration might also be affected. The present experiments record the phenotypic variations in the paired claw closer muscles of juvenile lobsters following regeneration.

Materials and Methods

Larval lobsters (*Homarus americanus*) were obtained from the Massachusetts State Lobster Hatchery on Martha's Vineyard and reared communally at the Marine Biological Laboratory, Woods Hole, Massachusetts, by methods described previously (Govind and Kent, 1982). Upon molting to the first post-larval or 4th stage, lobsters were reared individually in plastic trays containing pieces of oyster shells as substrate (Lang, 1975). On a daily basis, the animals were fed frozen brine shrimp and checked for molts to record their juvenile development.

Claws were removed by a gentle pinch, which elicited a reflex autotomy, resulting in the claw breaking off at a preformed fracture plane without much loss of blood. Both claws were so removed within 24 h after the animal had molted.

At the appropriate stages the regenerated paired claws were autotomized and prepared for histochemical examination of their muscles based on the stability of the myofibrillar ATPase enzyme to the pH of the incubating medium (Ogonowski and Lang, 1979). Thus, at pH 8, the enzyme is relatively stable in fast crustacean muscle, and hence these fibers stain more intensely in frozen cross-sections of the claw compared to slow muscle. The histochemically treated cross-sections of the claws were photographed, and the resulting photographs were used to calculate the percentage of fast and slow fibers. These calculations were made from the medial region of the claw, which provides the largest surface area, and hence is most representative of the entire muscle.

Results

Regenerated phenotype is retarded

We have previously shown that juvenile 4th and 5th stage lobsters reared with a substrate of oyster chips develop paired asymmetric (cutter/crusher) claws, while their counterparts reared without a substrate develop paired symmetric (cutter/cutter) claws (Lang *et al.*, 1978). Both rearing conditions were adopted for the present experiments. Thus, in the first experiment with oyster chips as a substrate, the development into asymmetric cutter and crusher type muscles is shown by plotting the percent of fast fibers in the paired original muscles (Fig. 1A). One of the muscles rapidly accumulates fast fibers to make up 90% of its mass and thus becomes a cutter type closer muscle. The slow fibers persist in a small (10%) ventral band (Fig. 2b). The contralateral muscle, which is the putative crusher, shows a more gradual loss of fast fibers, making up 10–20% by the 8th or 9th stage (Fig. 1A) and becoming zero by the 13th to 20th stage. The fast muscle in the putative crusher is restricted to a narrow central region (Figs. 2a).

Following autotomy of the paired claws in the 4th and 5th stages, the regenerated muscles in the 6th stage have a phenotype that is intermediate to the normal asymmetric condition (Fig. 1A). The fast muscle composition of the paired regenerated muscles is 57% and 44%, while that of the paired original muscles is 75% and 28%. The regional distribution of fast and slow fibers, however, is similar between original and regenerated claws in that the fast muscle is restricted to a central band while the slow muscle appears on either side (Fig. 2c, d). The regenerated muscle therefore appears to be retarded in its development. This retardation is temporary because the paired muscles show a normal phenotype by the 8th or 9th stage, despite loss of the paired claws in the 4th and 5th stages. In other words, recovery of the muscle phenotype following claw loss in the 4th and 5th stages occurs within 3 to 4 molts.

Loss of the paired claws for more prolonged periods, such as from the 4th to the 7th stage, successively results in the regenerated muscles in the 8th stage showing a retarded phenotype (Fig. 1A). The percent fast muscle in these regenerated muscles is 52% and 44% compared to the 90% of a normal cutter muscle. In both retarded muscles, the fast fibers are restricted to a central band (Fig. 3c, d) as compared to the normal cutter muscle in which the fast fibers occur over the entire area except for a small ventral band (Fig. 3b). The retardation in this case appears to be more permanent because paired muscles examined in the 10th stage still showed subnormal amounts of fast fibers, between 60–70%. Both muscles remained as putative cutter types as loss of the paired claws successively from the 4th to the 7th stage prevents the determination of bilateral asymmetry (Govind and Pearce, 1989). Recovery to 80–90% fast fiber composition was still not seen by the 13th to 15th juvenile stages, indicating that retardation of the muscle phenotype may be more permanent in these animals.

In the second experiment in which lobsters were reared without a substrate of oyster chips, the development of

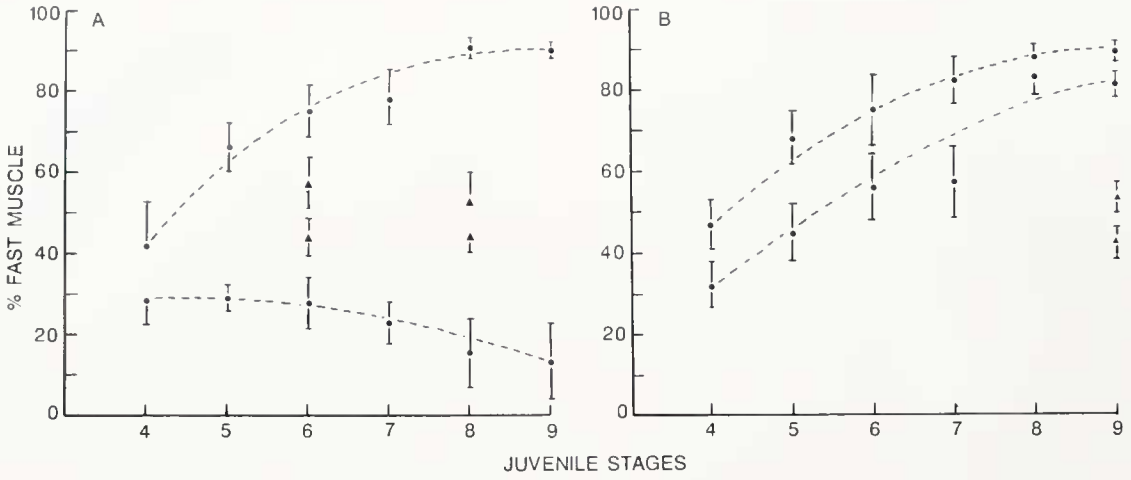

Figure 1. Percent composition of fast fibers in the paired claw closer muscles of original (circles) and regenerated (triangles) claws of juvenile lobsters reared with a substrate of oyster chips (A) and without a graspable substrate (B). For the regenerated condition, the paired claws were removed in all of the previous juvenile stages. Each point represents the mean and standard deviation of five animals. Curves fitting the points for each of the paired claw muscles is drawn by eye. The two curves in (B) were generated by arbitrarily assigning the muscle with the higher percentage of fast fibers to one group (upper curve) while its counterpart was assigned to the second group (lower curve).

the paired closer muscles into symmetric cutter types was followed by plotting the percent fast fibers in the paired original muscles (Fig. 1B). The paired muscles develop in a parallel fashion, accumulating fast fibers until these make up 80–90% of the total mass, and the remainder are slow fibers restricted to a ventral band. In other words, the paired muscles develop as typical cutter type muscles. In comparison, the regenerated phenotype in lobsters that had successively lost their claws from the 4th to the 8th stage, is distinctly retarded (Fig. 1B). The fast fibers in these regenerated muscles is between 40–50% compared to 80–90% in the original muscles. Moreover, as in the first experiment, the retarded condition persists for several subsequent stages at least until the 13th stage, which is as far as we proceeded in this experiment.

Regenerated phenotype shows mosaic pattern

As described above, the distribution of fast fibers in the paired closer muscles is restricted to a distinct central band. In the putative cutter muscle, this fast band during juvenile development rapidly enlarges to occupy almost the entire cross-sectional face, except for a slim ventral band (Figs. 2b, 3b). In the putative crusher, on the other hand, this central fast band gradually diminishes in size until it completely disappears (Figs. 2a, 3a). Throughout these developmental changes, the central band of fast fibers is homogenous and sharply delineated from the adjacent slow fibers.

The homogeneity of the fast band, however, was disrupted to various degrees in some of the regenerated muscles following autotomy of the paired claws. The least disruptive case was where slow fibers were occasionally interspersed among the fast fibers, especially along the lateral edges of the fast band (Fig. 2c, d; 3c, d). This gave the fast band a ragged edge, which was in contrast to its usual sharp edge. Much more disruptive cases involved considerable interspersing of slow fibers in the fast band (Fig. 4a, b), resulting in a distinct mosaic pattern.

Discussion

In a previous study (Kent *et al.*, 1989), we examined the phenotype of the regenerated closer muscle following claw loss in late juveniles and adults, when the claws and closer muscles were well differentiated into cutter and crusher types. In these cases, the regenerated claws and closer muscles resembled their predecessors with considerable fidelity. The present report examines the effect on the regenerate muscle phenotype following the loss of both claws in early juvenile stages when claw type is being determined (Emmel, 1908; Lang *et al.*, 1978) and fiber typing in the closer muscle is being expressed (Govind and Lang, 1978; Ogonowski *et al.*, 1980). Thus removal of paired claws successively either in the 4th and 5th stages or in the 4th through 7th stages resulted in a regenerated phenotype that resembled the undifferentiated condition in the normal 4th stage lobster. In the case where claw loss encompassed only the 4th and 5th stages, the regenerate muscle completes its differentiation into crusher and cutter types in subsequent stages. In the animals subjected to more prolonged claw loss (*i.e.*, from the 4th to the 7th

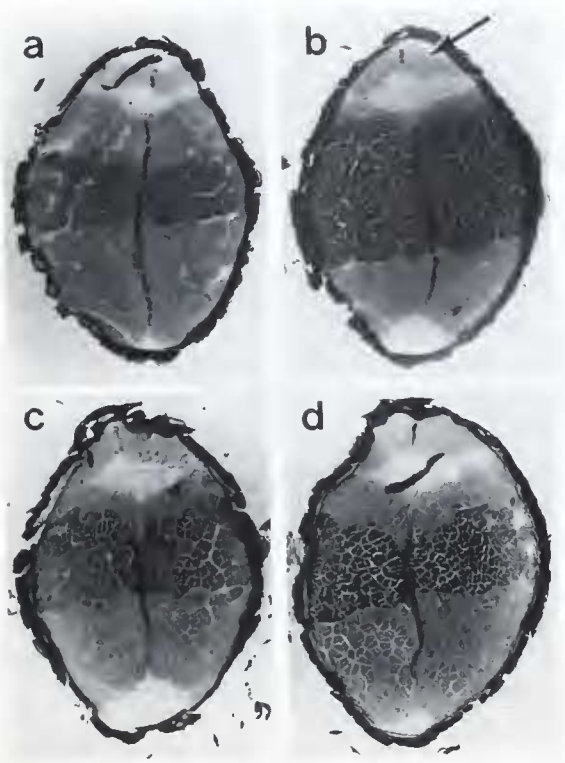

Figure 2. Cross-sections through the paired original (a, b) claws of a juvenile 6th stage lobster and through the paired regenerated (c, d) claws of another 6th stage lobster in which the claws had been removed in the 4th and 5th stages. Histochemical detection of myofibrillar ATPase activity shows fast fibers staining more intensely than slow, and hence the small, dorsally located opener muscle (arrow) is entirely slow while the large closer muscle occupying most of the cross-sectional area has a central band of fast fibers sandwiched dorsally and ventrally by slow fibers. The fast band varies considerably in size between the paired original muscles being narrow in the putative crusher muscle (a) and broad in the putative cutter muscle (b). In the paired regenerate muscles, however, the fast band is similar in size. Magnification 25×.

stage), however, the regenerate muscles have not completely differentiated into cutter types in the subsequent 3–5 stages. Thus the absence of the muscle during the critical juvenile stages results in regenerate phenotype being retarded. How long the muscle is retarded appears to depend on how often the claws are lost; when lost for two successive stages, the retardation is temporary but when lost over several successive stages, the retardation is more permanent.

A few of the regenerate muscles had slow fibers interspersed in the fast muscle band, giving rise to a mosaic distribution of these two types of fibers. This is an unusual distribution of fast and slow fibers in the closer muscle of lobsters as well as other decapod crustaceans. Thus, in the claw closer muscle of lobsters (Ogonowski *et al.*, 1980), crayfish (Govind and Pearce, 1985), snapping shrimps (O'Connor *et al.*, 1984), and hermit crabs (Stephens *et al.*, 1984), fast and slow muscle is regionally distributed; the fast fibers are restricted to a band in the central region. The closer muscle in the more anterior walking limbs in lobsters (Mearow and Govind, 1986) and hermit crabs (Stephens *et al.*, 1984) have a similar pattern. In no instance has a mosaic distribution of fast and slow fibers in the closer muscle been reported in the above mentioned species.

Apart from the closer muscles listed above containing discrete populations of fast and slow fibers, other muscles that have been examined are composed of a single fiber type, *e.g.*, the abdominal extensor and flexor systems that have separate fast and slow muscles in tailed crustaceans (Govind and Atwood, 1982). Consequently, the appearance of a mosaic distribution of fiber types is an uncommon finding among decapod crustaceans. That such a mosaic pattern occurs only in regenerated closer muscles and not in the originals suggests that the instructions for differentiating an entire muscle are not as robust as those

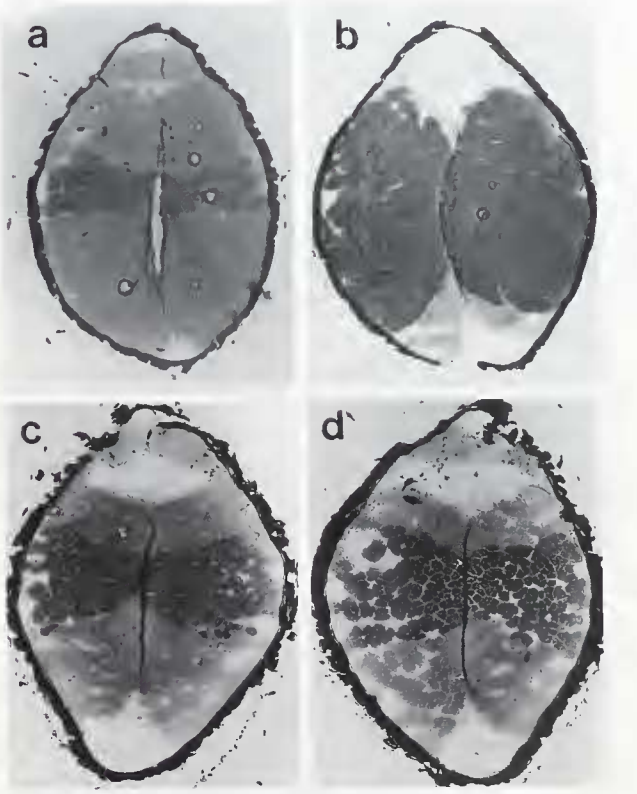

Figure 3. Cross-sections through the paired original (a, b) claws of a juvenile 8th stage lobster and through the paired regenerated (c, d) claws of another 8th stage lobster in which the claws had been removed in the 4th, 5th, 6th, and 7th stages. The proportion of fast fibers is highly asymmetric in the paired original closer muscles being restricted to a narrow central band in the crusher claw (a) but widespread in the cutter claw (b). In the paired regenerate muscles, however, the band of fast fibers is symmetric. Magnification 15×.

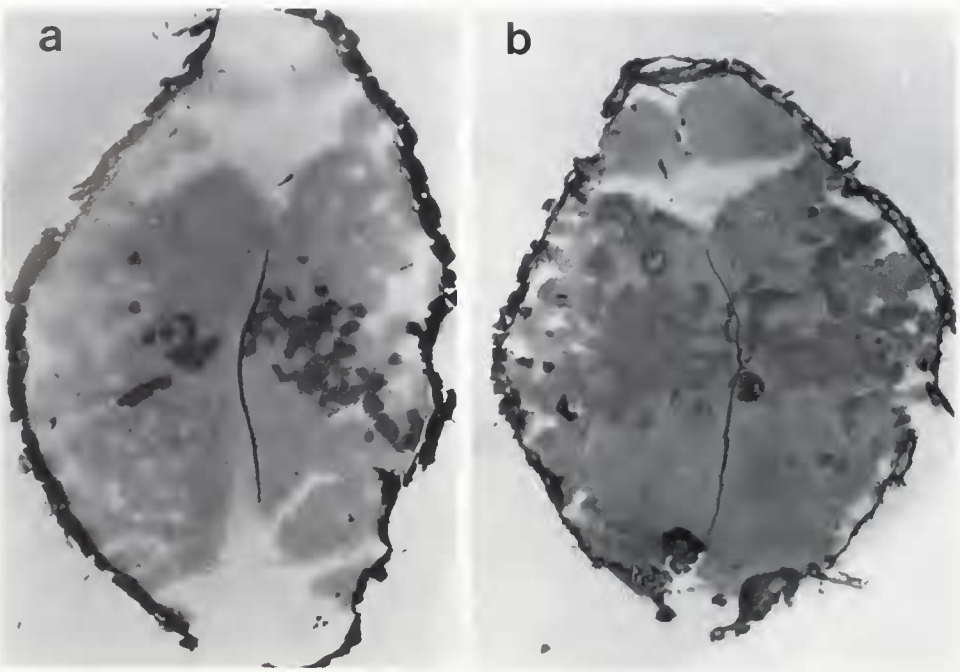

Figure 4. Cross-sections through the claws of two juvenile 8th stage lobsters (a, b) showing different degrees of interspersion of slow fibers (light staining) in the band of fast fibers (dark-staining), resulting in a mosaic appearance in the closer muscle. Magnification 35×.

for differentiating individual fiber types. Perhaps along similar lines is our observation that in the regenerated chelipeds of adult lobsters, the main limb nerve will often travel in a scattered, diffuse fashion rather than in discrete bundles. Although haphazard in appearance, the regenerated nerve contains the requisite motor and sensory neurons.

While a mosaic distribution of fiber types within a muscle occurs rarely in crustaceans, it is commonplace among vertebrates where individual limb muscles are innervated by a large number of motor neurons (Burke, 1981). Despite being randomly distributed within the muscle, fibers comprising a motor unit are of the same type. This has led to the suggestion that the innervating neuron regulates muscle fiber properties. Such neurotrophic regulation in the lobster claw closer muscle is unlikely as there are only two excitor neurons (Wiersma, 1961), both of which distribute to most of the muscle fibers (Govind and Lang, 1974).

Our findings also underscore the very robust nature of the regenerative capacity among juvenile lobsters. Apart from the slowing down in muscle differentiation and the occasional appearance of a mosaic distribution of fiber types, conditions that may be ameliorated, the regenerate muscle otherwise resembles its original counterpart. Thus, the loss of claws seen particularly in the early juvenile stages does not appear, in the long term, to impede the differentiation of a typical phenotype in the closer muscle.

Acknowledgments

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